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For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: COMPOSITIONS AND METHODS FOR THE ENHANCEMENT OF CHEMOTHERAPY WITH MICROBIAL CYTOTOXINS

(57) Abstract: Described herein is a microbial composition used to enhance anti-cancer drugs. Specifically, microbial compositions that comprise a part of or an entire microorganism having surface lectins specific to carbohydrate moieties on tumor surface combined with an oncolytic agent.

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US05/27187

A. CLASSIFICATION OF SUBJECT MATTER
IPC: A01N 63/00(2006.01)

USPC: 424/93.4
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 424/93.4

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Please See Continuation Sheet

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 6,190,657 B1 (PAWELEK et al.) 20 February 2001 (20.02.2001), abstract, column 2-6, summary of the invention, examples, and claims.	1-13
Y	US 6,645,946 B1 (KLYOSOV et al.) 11 November 2003 (11.11.2003), abstract, summary, examples, and claims.	1-13
Y	US 2004/0091503 A1 (SEGAL et al.) 13 May 2004 (13.05.2004), abstract, summary of the invention, table 1, [0143], [0536], example 18, and claims.	1-13
Y	MEY A. et al. The animal lectin Galectin-3 interacts with bacterial lipopolysaccharides via two independent sites. The Journal of Immunology. 1996, Vol. 156, pages 1572-1577, especially pages 1572, and 1576.	1-13
Y	PAN, Z.K. et al. A recombinant Listeria monocytogenes vaccine expressing a model tumour antigen protects mice against lethal cell challenges and causes regression of established tumours Nat. Med. 1995, Vol. 1, No. 5, pages 471-477, especially page 471.	1-13

☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search
02 July 2006 (02.07.2006)

Date of mailing of the international search report
08 AUG 2006

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Form PCT/ISA/210 (second sheet) (April 2005)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US05/27187

C. (Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	PLATT, D. et al. Modulation of the lung colonization of B16-F1 melanoma cells by citrus pectin. J. Natl. Cancer Inst. 1992, Vol. 84, No. 6, pages 438-442, especially page 438.	1-13
Y	KLYOSOV, A.A. et al. Preclinical studies of anticancer efficacy of 5-fluorouracil when coadministered with the 1,4-beta-D-galactomannan. Preclinica. September/October 2003, Vol. 1, No. 4, pages 175-183, especially page 175.	1-13
Y	PLATT, D. et al. Davanat- A modified branched galactomannan enhances chemotherapeutics: Reflections on manufacturing, pre-clinical studies and clinical trials. ABSTRACT No. 1 of papers, 227th ACS National Meeting, Anaheim, CA, USA, March 28- April 1, 2004; Publisher: American Chemical Society, Washington D.C.	1-13

Continuation of B. FIELDS SEARCHED Item 3:
EAST:
USPAT, USOCR, US-PGPUB, JPO, EPO, DERWENT.
STN:
CAPLUS, BIOSIS, MEDLINE, CONFSCI, EMBASE, WPIDS, PCTFULL, USPATFULL.
SEARCH STRATEGIES:
microb? or microorg? or bacter?
pertuss? or diptber? or clostrid? or lister?
cancer or neoplasm? or neoplast? or tumor?
chemother? or fluorourac? or drug? or agent?
galectin-3 or lectin? or mac-2 or galectin?
galactoman? or galactopyran? or davanat?

PATENT COOPERATION TREATY

REC'D 11 AUG 2006
WIPO PCT

From the
INTERNATIONAL SEARCHING AUTHORITY

To:
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PCT

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing (*day/month/year*)	08 AUG 2006
Applicant's or agent's file reference 13192-126PCT	FOR FURTHER ACTION See paragraph 2 below

International application No.	International filing date (*day/month/year*)	Priority date (*day/month/year*)
PCT/US05/27187	02 August 2005 (02.08.2005)	02 August 2004 (02.08.2004)

International Patent Classification (IPC) or both national classification and IPC

IPC: A01N 63/00(2006.01)
USPC: 424/93.4

Applicant

PRO-PHARMACEUTICALS INC.

1. This opinion contains indications relating to the following items:

- [X] Box No. I — Basis of the opinion
- [ ] Box No. II — Priority
- [ ] Box No. III — Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- [ ] Box No. IV — Lack of unity of invention
- [X] Box No. V — Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- [ ] Box No. VI — Certain documents cited
- [ ] Box No. VII — Certain defects in the international application
- [ ] Box No. VIII — Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA") except that this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notified the International Bureau under Rule 66.1*bis*(*b*) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of 3 months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

3. For further details, see notes to Form PCT/ISA/220.

Name and mailing address of the ISA/ US	Date of completion of this opinion	Authorized officer
Mail Stop PCT, Attn: ISA/US Commissioner for Patents P.O. Box 1450 Alexandria, Virginia 22313-1450 Facsimile No. (571) 273-3201	06 July 2006 (06.07.2006)	Satyendra K. Singh Telephone No. 571-272-8790

Form PCT/ISA/237 (cover sheet) (April 2005)

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US05/27187

Box No. I Basis of this opinion

1. With regard to the language, this opinion has been established on the basis of:

☒ the international application in the language in which it was filed

☐ a translation of the international application into _____, which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b)).

2. With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, this opinion has been established on the basis of:

a. type of material

☐ a sequence listing

☐ table(s) related to the sequence listing

b. format of material

☐ on paper

☐ in electronic form

c. time of filing/furnishing

☐ contained in the international application as filed.

☐ filed together with the international application in electronic form.

☐ furnished subsequently to this Authority for the purposes of search.

3. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table(s) relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

4. Additional comments:

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US05/27187

Box No. V Reasoned statement under Rule 43 *bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims 1-13	YES
	Claims NONE	NO
Inventive step (IS)	Claims NONE	YES
	Claims 1-13	NO
Industrial applicability (IA)	Claims 1-13	YES
	Claims NONE	NO

2. Citations and explanations:

Claims 1-13 meet the criteria set out in PCT Article 33(2), because the prior art does not teach or fairly suggest a therapeutic composition used to treat cancer comprising a cytotoxic microorganism and one or more chemotherapeutic agent, wherein said cytotoxic microorganism has a lectin that binds to a carbohydrate moiety on the surface pf a cancer cell, and wherein said composition synergistically inhibits tumor growth.

Claims 1-13 lack an inventive step under PCT Article 33(3) as being obvious over prior art cited by the examiner as follows:
PAWELEK et al (US 6,190,657 B1) teach microbial vectors (including bacteria, fungi, and protists) for the diagnosis and treatment of solid tumors including melanoma;
SEGAL et al (US 2004/0091503 A1) disclose various animal as well as microbial-derived lectin (including from bacteria such as Bordetella pertussis, Clostridium, Listeria, etc.) compositions and methods for modulating an immune response to an antigen;
KLYOSOV et al. (US 6,645,946 B1) disclose the use of a polysaccharide, galactomannan (GM) in combination with a chemotherapeutic agent (such as 5-fluorouracil, 5-FU; or adriamycin) in order to reduce toxic side effects of the agent used for the treatment of cancers (such as melanoma), and demonstrate an increased efficacy in the cancer treatment arising from a synergistic effect between the galactomannan and the therapeutic agent mixture (see column 4, in particular). In addition, KLYOSOV et al disclose the mode of action of such composition comprising a galactomannan through galactose-specific interactions at the surface of the target cells (i.e. tumor or neoplastic tissue; such as through galectins) and thus increasing the membrane fluidity and permeability of the target tumor cells, and therefore, effectively increasing the efficacy of the anticancer agent (see column 6, in particular).
MEY et al. (The J. Immunol., 1996) disclose the fact that animal lectin Galectin-3 interacts with bacterial lipopolysaccharides, the outer cell wall or capsular components of bacteria that is responsible for endotoxic shock, and cytotoxicity in the target cells through the release of inflammatory cytokines (such as tumor necrosis factor, TNF; see page 1572, in particular), thus suggesting the fact that lectins may play an important role in activation of cell death signaling pathways or apoptosis of target mammalian cells (i.e. tumor cells that have been known to overexpress carbohydrate-specific lectins on their surface).
PAN et al. (Nat. Med., 1995) demonstrate successful use of a recombinant Listeria spp. vaccine expressing a model tumor antigen in protecting mice against lethal tumor cell challenge and in reducing the size of pre-established macroscopic tumors.

Therefore, in view of the above cited prior art disclosures by the examiner, it would have been obvious to a person of ordinary skill in the art to modify the composition (and method of treatment) taught by KLYOSOV et al such that it includes attenuated, cytotoxic microbes (such as listeria, pertussis, clostridia, etc.) that can induce tumor cell death by interacting with galactose-specific lectins (such as galectin-3) expressed on the surface of tumor cells, and at the same time enhancing the efficacy of chemotherapeutic agent and reducing the toxicity or side effects of the anti-cancer agent used in the treatment of cancer. Thus, the invention as a whole lacks an inventive step.

Claims 1-13 meet the criteria set out in PCT Article 33(4), and thus claims 1-13 have industrial applicability because the subject matter claimed can be made or used in industry.